UNIVERSITI PUTRA MALAYSIA

IMMUNOMODULATORY EFFECTS OF NEWCASTLE DISEASE VIRUS STRAIN AF2240 ON HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS ACTIVATION AND CYTOLYTIC ACTIVITY

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MASTER OF SCIENCE

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

IMMUNOMODULATORY EFFECTS OF NEWCASTLE DISEASE VIRUS STRAIN AF2240 ON HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS ACTIVATION AND CYTOLYTIC ACTIVITY

By

LAM HAN YUEN

JUNE 2011

Chairperson: **Noorjahan Banu Mohamed Alitheen, PhD**

Faculty: **Biotechnology and Biomolecular Sciences**

Immunomodulator agent is a substance that can regulate the human immune system to reach therapeutic goal. In this study, Newcastle disease virus (NDV) was used as the immunomodulator to alter human immunity in order to replace current cancer therapies that cause severe side effects to cancer patients. The aim of this study is to examine the *in vitro* immunomodulatory effects of NDV strain AF2240 on human peripheral blood mononuclear cells (PBMC) proliferation, cytokines production and cytolytic effect on tumor cells. The cell proliferation of NDV-treated PBMC was

determined by BrdU cell proliferation assay. NDV virus titer 2 HAU was able to induce cell proliferation up to 30% indicating that low virus titer was sufficient to stimulate the human immune system. From the immunophenotyping results, the percentage of CD56 cells and cells expressed activating receptors (CD16 and NKG2D), which are normally expressed by natural killer (NK) cells, were increased. Therefore, NK cells might be the predominant activated effector cells in human PBMC. In addition, production of cytokines also revealed activation degree of PBMC, upon virus induction. After virus treatment for 72 hours, the level of cytokines, like IFN-γ, IL-2 and IL-12 were increased. These cytokines functioned to cause cell activation and proliferation and further augment the immune activities. In addition, the cytolytic effect on human tumor cells was determined by co-culturing NDV activated PBMC and tumor target cells. Results showed the activated human PBMC caused cytotoxicity towards human breast cancer, MCF-7 cells, by inducing apoptosis. Also, activated PBMC was cytotoxic on human liver cancer, HepG2 cells, and human leukemic, K562 cells. The findings showed that expression of perforin and granzyme B involved in cytolytic effect of activated PBMC on human tumor cells. In conclusion, NDV strain AF2240 was indicated as a potent immunomodulator to activate human PBMC that leads to cell proliferation, cytokines synthesis and enhancement of cytolytic effect on tumor cells.

Abstrak tesis yang dikemukakan kepada Senate Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

KESAN PEMODULASI-IMUN OLEH VIRUS PENYAKIT NEWCASTLE VIRUS STRAIN AF2240 TERHADAP PENGAKTIFAN SEL MONONUKLEAR DARAH PERIFERI MANUSIA DAN AKTIVITI SITOLITIK

Oleh

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Agen pemodulasi-imun merupakan satu bahan yang boleh mengawal sistem imun tubuh manusia untuk mencapai matlamat terapeutik. Dalam kajian ini, virus penyakit Newcastle (NDV) digunakan sebagai pemodulasi-imun untuk mengubah sistem immunisasi manusia bagi menggantikan terapi kanser terkini yang menyebabkan kesan samping yang serius untuk pesakit kanser. Tujuan kajian ini adalah untuk menguji secara in vitro kesan pemodulasi-imun strain NDV AF2240 ke atas proliferasi sel mononuklear darah periferi (PBMC) manusia, penghasilan sitokin dan kesan sitolitik pada sel tumor. Proliferasi sel PBMC yang dirawat dengan NDV

ditentukan melalui ujian proliferasi sel BrdU. Titer virus NDV 2 HAU mampu menyebabkan proliferasi sel sehingga 30% yang menunjukkan bahawa titer virus yang rendah sudah cukup untuk merangsangkan sistem imun tubuh manusia. Dari hasil Imunofenotip, peratusan sel CD56 dan sel-sel yang mengekspresikan reseptor pengaktifan (CD16 dan NKG2D), yang biasanya diekspresi oleh sel pembunuh semula jadi (NK), meningkat. Oleh itu, sel NK mungkin adalah sel efektor dominan yang diaktifkan dalam PBMC manusia. Selain itu, pengeluaran sitokin juga menunjukkan tahap pengaktifan PBMC, selepas induksi virus. Selepas dirawat dengan virus selama 72 jam, paras sitokin, seperti IFN-γ, IL-2 dan IL-12 meningkat. Sitokin ini berfungsi untuk menyebabkan pengaktifan sel dan proliferasi, juga meningkatkan kegiatan imun. Selain itu, kesan sitolitik pada sel tumor manusia ditentukan oleh ko-kultur PBMC yang diaktifkan oleh NDV dan sel tumor sasaran. Keputusan kajian menunjukkan PBMC manusia yang diaktifkan menyebabkan sitotoksik terhadap kanser payudara manusia sel MCF-7, dengan menginduksi apoptosis. Juga, PBMC yang diaktifkan adalah sitotoksik pada kanser hepar manusia, sel HepG2, dan leukemia manusia, sel K562. Keputusan kajian ini menunjukkan bahawa ekspresi perforin and granzyme B terlibat dalam kesan sitolitik oleh PBMC yang diaktifkan pada sel tumor manusia. Kesimpulannya, strain NDV AF2240 menunjukkan potensi sebagai pemodulasi-imun untuk mengaktifkan PBMC manusia, menyebabkan proliferasi sel, sintesis sitokin dan peningkatan kesan sitolitik pada sel tumor.

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I certify that a Thesis Examination Committee has met on 27th June 2011 to conduct the final examination of Lam Han Yuen on his thesis entitled "Immunomodulatory Effects of Newcastle Disease Virus Strain AF2240 on Human Peripheral Blood Mononuclear Cells Activation and Cytolytic Activity" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the degree Master of Science.

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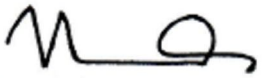

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The thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree Master of Science. The members of the Supervisor Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or other institutions.

LAM HAN YUEN

Date: 27 June 2011

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